

CLAIMS

WHAT IS CLAIMED IS:

1. A fluorogenic composition comprising a polypeptide backbone or a nucleic acid backbone joining two fluorophores of the same species whereby said
5 fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores.
2. The fluorogenic composition of claim 1, wherein said composition comprises a polypeptide backbone.
3. The fluorogenic composition of claim 2, wherein said polypeptide
10 backbone comprises a protease binding site ranging in length from about 2 to about 15 amino acids.
4. The fluorogenic composition of claim 2, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 8 amino acids.
5. The fluorogenic composition of claim 2, wherein said polypeptide
15 backbone ranges in length from about 4 to about 31 amino acids.
6. The fluorogenic composition of claim 2, wherein said composition is attached to a solid support.
7. The fluorogenic composition of claim 2, wherein said composition is
20 inside a mammalian cell.
8. The fluorogenic composition of claim 2, wherein said composition bears a hydrophobic group.
9. The fluorogenic composition of claim 8, wherein said hydrophobic
25 group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluoreneacetyl group, 9-fluoreneacetyl group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl

(Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-diaxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzoyloxycarbonyl (2-Cl-Z), 2-bromobenzoyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

10. The composition of claim 9, wherein said hydrophobic group is Fmoc.

11. The composition of claim 9, wherein said hydrophobic group is Fa.

12. The composition of claim 9, wherein said hydrophobic group is attached to the amino terminus of the molecule.

13. The fluorogenic composition of claim 1, wherein said composition comprises a nucleic acid backbone.

14. The fluorogenic composition of claim 13, wherein said nucleic acid backbone comprises a restriction site.

15. The fluorogenic composition of claim 13, wherein said nucleic acid backbone is self-complementary and forms a hairpin.

16. The fluorogenic composition of claim 13, wherein said nucleic acid backbone ranges in length from about 10 to about 100 nucleotides.

17. The fluorogenic composition of claim 13, wherein said nucleic acid backbone ranges in length from about 15 to about 50 nucleotides.

18. The fluorogenic composition of claim 13, wherein said composition is attached to a solid support.

19. The fluorogenic composition of claim 13, wherein said composition is inside a mammalian cell.

20. The fluorogenic composition of claim 13, wherein said composition bears a hydrophobic group.

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49. The method of claim 48, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 15 amino acids.

50. The method of claim 48, wherein said polypeptide backbone comprises a protease binding site ranging in length from about 2 to about 8 amino acids.

51. The method of claim 48, wherein said composition is attached to a solid support.

52. The method of claim 48, wherein said composition is inside a mammalian cell.

53. The method of claim 48, wherein said composition is inside a insect cell.

54. The method of claim 48, wherein said composition is inside a yeast cell.

55. The method of claim 48, wherein said composition bears a hydrophobic group.

56. The method of claim 48, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluoreneacetyl group, 9-fluoreneacetyl group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

57. The method of claim 56, wherein said hydrophobic group is attached to the amino terminus of the molecule.

67. The method of claim 66, wherein said nucleic acid backbone comprises a restriction site.

68. The method of claim 66, wherein said nucleic acid backbone is self-complementary and forms a hairpin.

5 69. The method of claim 66, wherein said nucleic acid backbone ranges in length from about 10 to about 100 nucleotides.

70. The method of claim 66, wherein said nucleic acid backbone ranges in length from about 15 to about 50 nucleotides.

10 71. The method of claim 66, wherein said composition is attached to a solid support.

72. The method of claim 66, wherein said composition is inside a mammalian cell.

73. The method of claim 66, wherein said composition is in solution.

15 74. The method of claim 66, wherein said composition bears a hydrophobic group.

75. The method of claim 74, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluoreneacetic acid, 9-fluoreneacetic acid, and 9-fluorene-1-carboxylic acid, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

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85. The method of claim 84, wherein said first molecule and said second molecule are selected from the group consisting of a receptor and a receptor ligand, an antibody and an antigen, a lectin and a carbohydrate, and a nucleic acid and a nucleic acid binding protein.
- 5 86. The method of claim 84, wherein said fluorophore is linked to said first molecule by a linker.
87. The method of claim 84, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.
88. The method of claim 84, wherein said fluorophores are selected from
10 the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.
89. A method of detecting a change in conformation or cleavage of a macromolecule, said method comprising:
 - i) providing a macromolecule having attached thereto two
15 fluorophores of the same species whereby said fluorophores form an H-dimer resulting in quenching of the fluorescence of said fluorophores; and
 - ii) detecting a change in fluorescence or absorbance of said fluorophores wherein a change in fluorescence or absorbance indicates a change in conformation or cleavage of said macromolecule.
- 20 90. The method of claim 85, wherein said macromolecule is selected from the group consisting of a polypeptide, a nucleic acid, a lipid, a polysaccharide, and an oligosaccharide.
91. The method of claim 85, wherein said macromolecule is attached to a solid support.
- 25 92. The method of claim 85, wherein said macromolecule is inside a mammalian cell.
93. The method of claim 85, wherein said macromolecule bears a hydrophobic group.

94. The method of claim 93, wherein said hydrophobic group is selected from the group consisting of: Fmoc, 9-fluoreneacetyl group, 1-fluorene-carboxylic group, 9-fluorene-carboxylic group, and 9-fluorenone-1-carboxylic group, benzyloxycarbonyl, Xanthyl (Xan), Trityl (Trt), 4-methyltrityl (Mtt), 4-methoxytrityl (Mmt), 4-methoxy-2,3,6-trimethyl-
5 benzenesulphonyl (Mtr), Mesitylene-2-sulphonyl (Mts), 4,4'-dimethoxybenzhydryl (Mbh), Tosyl (Tos), 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc), 4-methylbenzyl (MeBzl), 4-methoxybenzyl (MeOBzl), Benzyloxy (BzlO), Benzyl (Bzl), Benzoyl (Bz), 3-nitro-2-pyridinesulphenyl (Npys), 1-(4,4-dimethyl-2,6-diaxocyclohexylidene)ethyl (Dde),
10 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 2-chlorobenzyloxycarbonyl (2-Cl-Z), 2-bromobenzyloxycarbonyl (2-Br-Z), Benzyloxymethyl (Bom), t-butoxycarbonyl (Boc), cyclohexyloxy (cHxO), t-butoxymethyl (Bum), t-butoxy (tBuO), t-Butyl (tBu), Acetyl (Ac), and Trifluoroacetyl (TFA).

95. The method of claim 85, , wherein said fluorophores are linked to the macromolecule by linkers.

15 96. The method of claim 85, wherein said fluorophores have an excitation wavelength between about 310 nm and about 750 nm.

97. The method of claim 85, wherein said fluorophores are selected from the group consisting of carboxytetramethylrhodamine, carboxyrhodamine-X, carboxyrhodamine 110, diethylaminocoumarin, and carbocyanine dyes.

20 98. The method of claim 85, wherein said contacting is in a histological section.

99. The method of claim 85, wherein said contacting is in a cell culture.

100. The method of claim 85,, wherein said contacting is contacting a seeded or cultured adherent cell.

25 101. The method of claim 85,, wherein said contacting is in a cell suspension derived from a biological sample selected from the group consisting of a tissue, blood, urine, saliva, lymph, biopsy.

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